The Influence of Latent Viral Infection on Rate of Cognitive Decline over 4 Years

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OBJECTIVES: To examine whether cytomegalovirus (CMV) and herpes simplex virus type-1 (HSV-1) are associated with cognitive decline over a 4-year period and to assess whether C-reactive protein (CRP) modifies these relationships.

DESIGN: Prospective cohort study over a 4-year period.

SETTING: Community-dwelling elderly population.

PARTICIPANTS: The sample was a subset (1,204/1,789) of participants in the Sacramento Area Latino Study on Aging (SALSA) aged 60 to 100.

MEASUREMENTS: Participants were screened annually over a 4-year period for cognitive function and episodic memory. Cognitive function was assessed using the modified Mini-Mental State Examination, and episodic memory was assessed using a word list-learning test of delayed recall. Baseline serum samples were assayed for levels of immunoglobulin G antibodies to CMV and HSV-1 and for levels of CRP.

RESULTS: There was a significantly higher rate of cognitive decline over the 4-year period in subjects with the highest CMV antibody levels at baseline than in individuals with the lowest levels ($\beta = -0.053$, standard error = 0.018; $P = .003$), after controlling for age, sex, education, income, and chronic health conditions. There was no association between HSV-1 antibody levels and cognitive decline. CRP did not modify the relationship between viral antibody levels and cognitive decline.

CONCLUSION: This is the first study to show that individuals with higher levels of antibody to CMV experience a more-rapid rate of cognitive decline than those with lower levels. Understanding the mechanisms by which CMV influences cognition may aid development of intervention strategies targeting infection, viral reactivation, and immune response over the life course. **J Am Geriatr Soc 54:1046–1054, 2006.**

Key words: herpes; dementia; rate of cognitive decline; elderly; community

Cognitive impairment and dementia are important causes of increased morbidity and mortality in older people. As the aging population continues to grow in the United States, the healthcare needs associated with treating and caring for elderly individuals with dementia are projected to pose significant public health and economic burdens.[1,2] Researchers agree that it is critical to diagnose dementia at an early stage to implement prevention efforts and maximize treatment benefits.[3] Studies have implicated biological inflammatory markers, such as C-reactive protein (CRP) and cytokines, as predictors of cognitive decline.[4–6] Although the identification of such inflammatory markers has helped unveil the biological pathways that influence dementia risk, limited information is available regarding the pathophysiological mechanisms that modulate changes in the level of these markers.

One potential ubiquitous early-life environmental exposure that may act as a proinflammatory factor is latent infection with herpesviruses, such as cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1). CMV and HSV-1 are extremely prevalent in older people, and identification of these viruses in areas of the brain of individuals affected by Alzheimer's disease have led to the hypothesis that herpesviruses are causal agents or promoters of dementia.[7] The pathways by which herpesviruses may influence dementia are not well understood. It is possible that stress-induced viral reactivation may lead to immune-related inflammatory cascades and cytokine accumulation, resulting in direct damage to neurons.[8] It has been suggested that, even when herpesviruses are in a latent state,

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A published abstract of the initial findings, "Novel Infectious Biomarkers of Cognitive Impairment," was presented at the Alzheimer's Association International Prevention of Dementia Conference, June 18–21, 2005, Washington, DC. In addition, we were invited to submit a brief report to the journal *Research and Practice in Alzheimer's Disease and Cognitive Decline* on the findings presented at the Alzheimer's Association International Prevention of Dementia Conference. This four-page invited brief report is currently under review and has limited overlap with the findings reported in this study.

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inflammatory processes are ongoing and may be enhanced by age-related changes in the immune system. For example, latent CMV has been shown to be highly immunostimulatory in older people and is associated with increased CMV-specific CD8+ T-cell proliferation, which may result in heightened levels of circulating inflammatory cytokines.[9]

Given the potential pathways between infection, inflammation, and cognitive decline, it was hypothesized that latent viral infections directly influence cognition and that CRP modulates the relationship between herpesviruses and cognitive decline. The specific aims of this study were to examine whether levels of viral antibodies to CMV and HSV-1 influenced cognitive decline over a 4-year follow-up period and to assess whether CRP modifies the effect of viral antibody levels on cognitive decline.

METHODS

Study Population

The participants were derived from the Sacramento Area Latino Study on Aging (SALSA). SALSA is a large, ongoing, prospective cohort study of Mexican Americans living in the community who were aged 60 to 100 at baseline in 1998/99. A subsample of participants (N = 1,204) from the overall SALSA cohort (N = 1,789) with available blood samples and who had at least two sequential follow-up visits starting from baseline were included in the analyses. The details of the SALSA study have been discussed previously, and the institutional review board at the University of Michigan approved all analyses.[10]

Study Design

Baseline data collection for the SALSA began in 1998 with a 2-hour interview at participants' homes. Each year, participants were screened for cognitive functioning using the modified Mini-Mental State Examination (3MSE) and for episodic memory using a word list-learning test of delayed recall (DEL-REC).[10] Those falling below the education- and age-adjusted 20th percentile on either of these tests were referred for further neuropsychological tests, a clinical examination, and expert adjudication of dementia and cognitive impairment diagnoses. A subsample, including 20% of subjects with values above the 3MSE or DEL-REC cutpoints, underwent further evaluations aimed at determining the sensitivity of screening tests, as described elsewhere.[10] Lifestyle, health, and demographic factors were collected annually during the participant interviews. Information on education, socioeconomic status of the household, medical history, and duration and date of diagnoses for 35 health conditions were gathered. Direct clinical evaluations were used to measure blood pressure and weight/height and waist/hip ratios.

Laboratory Analyses

The laboratory staff was unaware of the diagnostic classification of the test samples. A commercially available enzyme-linked immunosorbent assay (ELISA) immunoglobulin G (IgG) detection system (Wampole Laboratories, Princeton, NJ) was used to analyze the level (expressed as optical density units) of IgG reactive to HSV-1 and CMV in aliquots of continuously frozen (-70°C) serum samples. Following manufacturer instructions, samples were assessed for the level of IgG antibody signal as expressed by the mean optical density value. The sensitivity and specificity of the assay for HSV-1 are reported to be 97.1% and 96.8%, respectively, and for CMV, the sensitivity and specificity are reported to be 96.4% and 93.3%, respectively. Each 96-well commercial ELISA kit contained controls and internal calibrations.

CRP was analyzed using the CRP Ultra Wide Range Reagent Kit latex-enhanced immunoassay (Equal Diagnostics, Exton, PA). This is a widely used in vitro diagnostic test for the quantitative determination of CRP in human serum. Manufacturer's instructions were followed, and CRP levels were determined using an automated analyzer and a prepared calibration curve. Using the highly sensitive method, the kit provides a range of measurable CRP from 0.05 mg/L to 80.0 mg/L. Results greater than 80.0 mg/L were diluted, retested, and multiplied by the dilution factor.

Statistical Analyses

For descriptive demographic variables shown in Tables 1 and 2, *t* tests were used to compare means, and chi-square or Fisher exact tests to compare proportions. Mixed models were used to estimate the effect of viral antibody levels measured at baseline on changes in cognitive functioning over a 4-year period.[11] The measures of cognitive functioning were the 3MSE and DEL-REC scores at baseline and each of a maximum of four follow-up measurements. Because the distribution of 3MSE scores was negatively skewed, a natural logarithmic transformation was applied to this outcome to meet the model assumption of normality.[12] All models presented with 3MSE as the outcome used the transformed version of this measure. In addition, measured CRP levels in mg/L were skewed and were therefore log transformed (lnCRP) to maintain a normal distribution.

In the initial mixed-model analysis, the independent influence of several potential covariates that have been identified as risk factors for dementia was explored.[13] These covariates were age, sex, education, income, number of health conditions reported at baseline (diabetes mellitus, hypertension, myocardial infarction, angina pectoris, heart failure, atrial fibrillation, rheumatic fever/heart valve problem, cancer, and stroke), smoking, body mass index, CRP levels, Center for Epidemiologic Studies Depression Scale scores,[14] and hetero- or homozygosity for apolipoprotein E-4 genotype (ApoE-4). Each covariate was assessed in repeated-measures mixed models representing decline in 3MSE. In addition, the relationship between each of these potential covariates and the main predictor variables (i.e., baseline CMV and HSV-1 antibody levels) were examined using linear regression analyses. Consistent with cutoffs commonly used in the epidemiological literature, only covariates that were significantly associated with baseline cognitive scores at $\alpha = 0.05$ level and with cognitive decline (i.e., covariate by time) at $\alpha = 0.10$ level were included. The covariates that exhibited statistical significance using these criteria were age, sex, education, income, and number of health conditions at baseline.

Modifications were made to the mixed-model covariance structure to obtain better fitting models, as indicated by a lower Akaike Information Criterion. Based on the

Table 1. Descriptive Statistics for the Sacramento Area Latino Study on Aging Cohort Analysis Sample (N = 1,204)

Characteristic	Value
Age at baseline, mean ± SD*	70.3 ± 6.8
Female, %	58
Place of birth Mexico or other Latin American country, %†	46.3
Number of baseline health conditions, mean ± SD	1.3 ± 1.1
Apolipoprotein-E4 positive‡, %	5.7
Education, years, %§	
0–3	28.7
4–11	37.9
≥12	33.4
Income per month, $, %§	
<1,000	40.5
1,000–1,999	31.9
≥2,000	27.6
3MSE score, mean ± SD (median)‖	
Baseline	85.7 ± 13.0 (89)
Follow-up year 1	85.4 ± 15.2 (90)
Follow-up year 2	89.6 ± 11.4 (93)
Follow-up year 3	88.9 ± 12.5 (93)
Follow-up year 4	87.1 ± 15.5 (92)
DEL-REC score, mean ± SD¶	
Baseline	8.6 ± 3.1
Follow-up year 1	8.0 ± 3.3
Follow-up year 2	8.8 ± 2.6
Follow-up year 3	7.9 ± 2.8
Follow-up year 4	8.7 ± 3.1

* The mean age of the analysis sample was significantly younger than of those excluded (76.4 vs 77.3, $P = .01$).
† A smaller percentage of individuals in the analysis sample were born in Mexico than of those excluded (46.3% vs 60.7%, $P < .001$).
‡ Information on apolipoprotein-E4 status was not available for 80 individuals.
§ Education and income levels of the analysis sample were significantly higher than of those excluded (all $P < .001$).
‖ For Modified Mini-Mental State Examination (3MSE) scores, the mean and median are presented, because these values were not normally distributed. Individuals in the analyses sample had significantly higher 3MSE scores at baseline ($P < .001$) and at Year 3 ($P = .04$) than those excluded.
¶ Delayed Recall Word List (DEL-REC), scores were significantly higher in the analysis sample at baseline ($P < .001$) and Year 4 ($P = .04$) than for those excluded.
SD = standard deviation.

Table 2. Proportion Seropositive for Cytomegalovirus (CMV) and Herpes Simplex Virus Type-1 (HSV-1) and Levels of C-Reactive Protein (CRP)

Biomarker	Women (n = 698)	Men (n = 506)	Total Sample (N = 1,204)
Virus immunoglobulin G antibody levels*			
CMV	97.56	95.26	96.59
HSV	98.57	97.63	98.17
CRP levels (mg/L)†			
Low (<1)	12.63	25.69	18.12
Medium (1–3)	26.40	33.20	29.26
High (>3)	60.98	41.11	52.62

* Based on enzyme-linked immunosorbent assay clinical cutpoints for seropositivity.
† Cutpoints based on Centers for Disease Control and Prevention and the American Heart Association.[16]

modifications, all models presented assumed a first-order auto-regressive covariance structure for the within-subject error and contained a random intercept allowing variation in baseline measures of cognition. Also, to account for the curvilinear pattern of change in cognitive functioning over time as measured using the 3MSE, all models for this outcome contained a quadratic term representing the year (time) of cognitive test scores. Mixed models are presented in two forms: (1) treating CMV and HSV-1 antibody values as continuous variables, which assumes a linear relationship between viral IgG antibody levels and decline in cognition over time, and (2) a transformation of CMV and HSV-1 antibody values into quartiles, where the first quartile is considered the referent group for comparison with the second, third, and fourth quartiles of viral antibody levels. This form of the model allows an assessment of potential nonlinear relationships and provides a means of assessing a dose–response effect of viral antibody levels on rate of cognitive decline. Details on the application of mixed models to cognitive decline have been described previously.[15]

The unadjusted mixed models for assessing the relationship between viral antibody level and cognitive decline included a term for the viral antibody value, time (since baseline), and the interaction of the viral antibody value with year. The next model (initial model) contained the same variables as the unadjusted model with the addition of age and sex and their interaction with time. The final models contained all of the variables in the initial model along with all other significant covariates (education, income, and number of baseline health conditions).

To examine whether lnCRP modified the effect of viral antibody levels on global cognitive decline (3MSE), unadjusted and adjusted mixed models with three-way interaction terms were fitted. For example, the unadjusted effect modification model for CMV and lnCRP included the following terms: CMV (antibody levels as a continuous value), CRP, time, CMV by time, lnCRP by time, lnCRP by CMV, and CMV by CRP by time. Analyses were conducted using SAS Version 9.1 (SAS Institute, Inc., Cary, NC) with the corresponding commands for the MIXED model procedure.

RESULTS

Demographic Characteristics

The demographic characteristics of subjects who were included in the analyses (N = 1,204) are shown in Table 1. Using the clinical cutpoint designated by the ELISA test kits, 97% of the study subjects showed signs of prior infection with CMV, and 98% showed signs of prior infection with HSV-1 (Table 2). Levels of CMV IgG antibodies were significantly higher in women than men (both $P < .001$), but there was no significant difference in levels of HSV-1 (Figure 1). Most of the participants had CRP levels that have been considered to indicate high risk for cardiovascular conditions; CRP levels varied by sex.[16] There were significant, but modest, linear associations between antibody levels of HSV-1 and CMV ($\beta = 0.147$, $P < .001$), and CMV

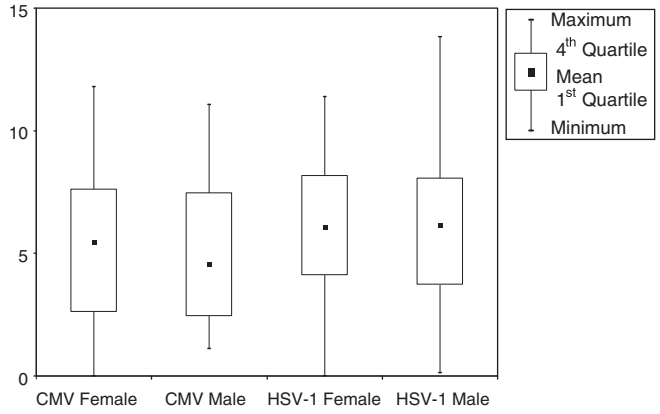

Figure 1. Quartile distribution of cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1) immunoglobulin G (Ig6) antibody values. The box-plots show the IgG antibody optical density units as measured using enzyme-linked immunosorbent assay.

and lnCRP ($\beta = 0.113$, $P < .023$), controlling for age, sex, and number of health conditions. There was no significant linear association between lnCRP and HSV-1 antibody levels ($\beta = 0.050$, $P > .26$).

Viral Antibody Levels and Rate of Cognitive Decline

A one-unit increase in CMV IgG antibody level was associated with a significant decline in 3MSE score over the 4-year period ($\beta = -0.274$, standard error $= 0.023$; $P < .001$) controlling for age, sex, education, income, and chronic health conditions. This same trend was observed for the quartile analyses. Tables 3 and 4 show the initial and final mixed-model effect estimates for quartiles of viral antibody levels on annual rate of cognitive decline as measured using the 3MSE at each of the five yearly assessments (i.e., baseline, Year 1, Year 2, Year 3, Year 4). As denoted by the significant follow-up-time parameter estimates in Tables 3 and 4, subjects showed an increase in 3MSE scores over the 4-year study period, indicating a moderate 3MSE practice effect over time.

Individuals with CMV antibody levels in the upper quartiles (4th and 3rd) had a more rapid rate of cognitive decline over the 4-year period than individuals in the lowest category of CMV antibody levels (1st quartile), controlling for all covariates (age, sex, education, income, and health conditions). There was an increase in the magnitude of the difference, with each quartile compared with the referent group.

In contrast to CMV, the HSV-1 antibody levels did not significantly influence rate of cognitive decline over the 4-year period (Table 4). In addition, neither CMV nor HSV-1 antibody levels were significantly associated with baseline DEL-REC scores in final models (data not shown, all $P > .05$) or declines in the DEL-REC over the 4-year period (data not shown, all $P > .10$).

Effect Modification by CRP on Cognitive Decline

There was no significant interaction between increased viral antibody levels and lnCRP on rate of cognitive decline (i.e., CMV by lnCRP by time), controlling for age, sex, health conditions, education, and income (Table 5). In the final effect-modification model for CMV, the relationship between higher CMV antibody levels and rate of cognitive decline (CMV by time) remained significant, regardless of the inclusion of variables representing lnCRP, its interaction with time, the lnCRP-by-CMV interaction, the three-way interaction term (lnCRP by CMV by time), and covariates. Effect modification with DEL-REC scores as an outcome was not assessed, because neither CMV nor HSV-1 was independently associated with decline in these test scores in final models.

DISCUSSION

This was the first study to examine the relationship between herpesvirus antibody levels and rate of cognitive decline over a 4-year period in community-dwelling elderly individuals in the United States. The strengths of this novel

Table 3. Relationship Between Cytomegalovirus (CMV) Antibody Level and Cognitive Decline

	Model 1*	Model 2†
Parameter	Mixed-Model β Effect Estimate (Standard Error)	
Time	0.324 (0.037)‡	0.302 (0.025)‡
CMV quartile		
4	−0.019 (0.057)	0.098 (0.057)
3	−0.164 (0.057)§	−0.023 (0.056)
2	−0.037 (0.056)	0.036 (0.055)
1	Reference	—
Interaction between CMV quartile and time		
4 by time	−0.064 (0.025)‖	−0.053 (0.018)‖
3 by time	−0.043 (0.025)‖	−0.037 (0.017)‖
2 by time	−0.021 (0.025)	−0.013 (0.017)
1 by time	Reference	—

Note: Results compare the effect of each quartile of CMV immunoglobulin G antibody level with the lowest quartile (1) on Modified Mini-Mental State Examination scores over the 4-year period.

* Adjusted for age, sex, and their interaction with time.

† Adjusted for age, sex, their interaction with time, education, income, and number of baseline health conditions.

$P <$ ‡.001; § .05; and ‖.10 for interaction terms.

Table 4. Relationship Between Herpes Simplex Virus Type 1 (HSV-1) Antibody Level and Cognitive Decline

Parameter	Model 1*	Model 2†
	Mixed-Model β Effect Estimate (Standard Error)	
Time	0.299 (0.038)‡	0.278 (0.026)‡
HSV-1 quartile		
4	−0.111 (0.056)	0.026 (0.056)
3	−0.078 (0.056)	0.013 (0.056)
2	−0.094 (0.056)	−0.012 (0.056)
1	Reference	—
Interaction between HSV-1 quartile and time		
4 by time	−0.018 (0.025)	0.003 (0.017)
3 by time	0.013 (0.025)	0.011 (0.018)
2 by time	0.021 (0.025)	0.010 (0.017)
1 by time	Reference	—

Note: Results compare the effect of each quartile of HSV-1 immunoglobulin G antibody level with the lowest quartile (1) on Modified Mini-Mental State Examination scores over the 4-year period.
* Adjusted for age, sex, and their interaction with time.
† Adjusted for age, sex, their interaction with time, education, income, and number of baseline health conditions.
‡ Significant at $P < .001$.

study include the rigorous, widely accepted, standardized methods for ascertaining cognitive functioning; assessment and control of numerous potential covariates; and use of a prospective study design and population-based cohort. In addition, mixed-model analyses were used to assess potential dose–response relationships and to model corresponding trajectories of cognitive decline over a 4-year period. The results suggest that high CMV IgG antibody levels are an important marker of cognitive decline in older people, even after controlling for major risk factors such as age, education, sex, and chronic health conditions. A dose–response relationship was observed in which each increase in quartile of CMV antibody level resulted in a more rapid rate of cognitive decline. The general inflammatory marker CRP in the study population did not modify this relationship.

CMV and Cognitive Decline

The findings of an association between high CMV IgG antibody levels and cognitive decline are consistent with one previous epidemiological study that identified a relationship between herpesvirus coinfection and cognitive impairment in elderly Finnish patients with underlying vascular disease.[17] The study showed that Finnish subjects who tested positive for serum antibodies to CMV, HSV-1, and HSV-2 at baseline had twice the risk of decline in cognitive functioning scores after 1 year as those who did not test positive.[17] Exposure status was measured according to the presence or absence of IgG antibodies to each virus and combined seropositivity. The use of seropositivity (i.e., positive or negative for CMV or HSV IgG antibodies or both) as a predictor can result in small comparison groups of nonexposed individuals, given the high prevalence of prior infection with HSV-1 and CMV in the general population.[18–20] The use of seropositivity status as an exposure variable has been shown to result in instability of regression estimates due to random variability from small cell sizes and residual confounding.[18] In contrast to the previous study,[17] the current study used antibody levels as continuous and quartile-transformed exposure measures to assess linear and potential dose–response relationships, as well as to overcome analytical limitations associated with extremely prevalent predictor variables. The findings regarding CMV

Table 5. Effect Modification by Log_{10} C-Reactive Protein (lnCRP) on Cognitive Decline (3MSE Score)

Parameter (Quartile)	Model 1*	Model 2†
	Mixed-Model β Effect Estimate (Standard Error)	
Effect modification of CMV by lnCRP on cognitive decline		
CMV by time	−0.014 (0.003)‡	−0.010 (0.003)‡
CMV by lnCRP by time	0.001 (0.003)	0.001 (0.003)
Effect modification of HSV-1 by lnCRP on cognitive decline		
HSV-1 by time	−0.003 (0.004)	−0.002 (0.004)
HSV-1 by lnCRP by time	−0.001 (0.003)	0.001 (0.003)

* Adjusted for time, time by time, virus (CMV or HSV-1), lnCRP, virus by lnCRP, virus by time, lnCRP by time, and virus by lnCRP by time.
† Adjusted for all variables in Model 1 plus age, sex, education, income, and number of baseline health conditions.
Results represent a one-unit increase in herpes virus immunoglobulin G antibody levels (measured in optical density units) on modified Mini-Mental State Examination (3MSE) scores over the 4-year period.
‡ Significant at $P < .10$.
CMV = cytomegalovirus; HSV-1 = herpes simplex virus type 1.

antibody levels and cognitive decline were robust even after controlling for several significant covariates that have been identified as risk factors for cognitive decline, including age, education, and numerous health conditions.

It has been shown that acute symptomatic infection in elderly hospitalized individuals, such as urinary tract infection, can trigger transient changes in cognition and attention, resulting in episodes of delirium.[21] These bouts of delirium are a major risk factor for subsequent functional decline and dementia diagnoses.[22,23] In contrast to acute systemic infection, CMV is a lifelong latent infection that generally does not cause clinical symptoms in healthy individuals.[19] It is possible that latent CMV plays a role in cognitive decline via immune system modulation and associated inflammatory cytokine cascades rather than direct pathological effects attributed to active infection. Novel research findings have shown that latent CMV infection in older people is an important component of a set of immunological parameters designated the "immunological risk phenotype."[24] This set of immunological markers, including latent CMV infection, high cluster of differentiation (CD)8 cells, low CD4 cell percentages, and poor T-cell proliferation, is predictive of mortality in healthy elderly individuals.[25]

Latent CMV is present in CD34+ bone marrow precursor cells and during differentiation of these precursors, CMV may chronically stimulate the immune system.[26] Several studies have now reported that there is large clonal expansion of CMV-specific CD8+ T-cells in older people and that the expansion of these clones are not a consequence of deteriorating health, comorbidity, or a general loss of immune control over the virus.[9,25,27–30] The expansion of CMV-specific clones reduces the availability of CD8+ T-cell carrying receptors that are specific for pathogens or foreign antigens other than CMV, thereby limiting the capacity of the immune system to mount an efficient response.[31] For example, one study reported that a reduced immune response to influenza vaccination was observed in elderly individuals who tested seropositive for latent CMV.[31] Moreover, they showed that high levels of CMV IgG antibody are strongly correlated with increases in serum cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 in older people.[31] CMV-specific CD8+ T-cells also have the ability to produce interferon (FN)-γ.[9] Thus, an accumulation of CMV-specific CD8+ T-cells may lead to an increase in several circulating inflammatory cytokines, including TNF-α, IFN-γ, and IL-6. This increased chronic peripheral cytokine concentration and reduced repertoire of T-cells with specificity for pathogens other than CMV is likely to influence pathophysiological changes associated with cognitive impairment and dementia.[32]

The finding of a dose–response relationship between CMV IgG antibody levels and cognitive decline is further evidence for an infection-related immunological pathway. Research is needed to assess whether cytokines, such as IL-6 and TNF-α, modify the relationship between CMV and cognitive decline, because these markers may increase with heightened levels of CD4+ CMV-specific T-cells associated with the aging process.[9] Recently, a cross-sectional study showed a significant association between CMV infection and frailty that was strongly modified by increased IL-6 levels.[33] Their findings suggest that proinflammatory cytokines may be an important modulator of latent CMV infection in older people in relation to declines in physical functioning.[33]

HSV-1 and Cognitive Decline

Although a few autopsy-based case-control studies have reported that HSV-1 deoxyribonucleic acid (DNA) is found in a higher proportion of the brains of people with Alzheimer's disease than in age-matched controls,[34–36] the majority of these autopsy studies report no association between the presence of the viral DNA and dementia.[37–43] The results of the current study do not support a relationship between HSV-1 and cognitive decline.

Studies have implicated ApoE-4 as a potential susceptibility genotype for infection with various pathogens, including HSV-1, human immunodeficiency virus, and malaria.[44] Several case-control studies have indicated that individuals who are positive for the ApoE-4 allele and for HSV-1 DNA in the brain have a greater risk of Alzheimer's disease than those who are ApoE4 positive but do not have virus present in the brain or than those without either the virus or the ApoE-4 allele.[38–41] This suggests a potential interaction between HSV-1 and ApoE-4 and dementia risk.[7] It was not possible to identify any studies that have examined interaction between CMV and ApoE-4 and dementia risk. As a consequence of the limited sample size of individuals positive for ApoE-4 (5.7%), there was insufficient power to explore interactions between E-4 and the viral antibody markers in this study population. Further research is needed to assess potential gene–virus interactions with cognitive decline in large population-based prospective studies.

Effect Modification of Viral Cognitive Trajectories According to CRP

Two studies have reported a relationship between high CRP levels and cognitive decline.[4,5] In addition, several researchers have reported an association between high CRP and risk of dementia.[45–47] The specific mechanisms by which CRP influences cognitive impairment are still unclear. In an in vitro study, CRP has been shown to cause toxicity to neuronal cells,[48] and studies have identified circulating CRP in the brains of people with Alzheimer's disease.[49,50] Although CRP has been shown to be a more-sensitive marker of bacterial than viral infection, it also fluctuates with the clinical course of several viral infections, including CMV.[51,52] Nevertheless, there was no indication of an interaction between CRP and viral antibody levels on changes in cognitive scores over the 4-year period in the study population. The association between high CMV antibody levels and rate of decline in 3MSE scores was robust, regardless of the inclusion of CRP levels in the interaction models. This suggests that the effect of high CMV antibody levels on rate of cognitive decline is independent of general inflammatory processes associated with increased CRP. Further study on the pathophysiological pathways by which CMV influences cognitive decline is warranted.

Limitations

There are potential limitations associated with use of viral antibodies as a marker. For example, high levels of herpes-

virus IgG antibodies may indicate recent primary infection, but this is unlikely in the study population, because infection with HSV-1 and CMV commonly occurs at young ages. (Eighty percent to 90% of elderly populations are infected.)[19,20] There are few data assessing CMV infection in population-based studies of older Mexican Americans. The studies that are available have been conducted in Mexican Americans in other age ranges.[53,54] These studies generally report a higher prevalence of CMV infection in Mexican Americans than in non-Hispanic whites. The prevalence of prior infection with HSV-1, which is a virus that is similar to CMV in terms of transmission, exposure, and latency properties, has been shown to be significantly higher in U.S. Hispanics aged 60 and older than in non-Hispanic whites participating in the National Health and Nutrition Examination Survey III.[20] The seropositivity estimates for HSV-1 observed in the current study are consistent with these nationally representative prevalence estimates of HSV-1 in Hispanic individuals aged 60 and older.

Given that the prevalence of CMV infection is found to be higher in Mexican Americans, it is also possible that reactivation and reinfection may be more common over the life-course. Such a pattern of infection and reactivation could lead to an increase in circulating levels of inflammatory cytokines as an adult. CMV has been shown to induce IL-6 and TNF-α from macrophages and microglial cells.[55] Information was not available on levels of inflammatory cytokines to assess potential correlations with high CMV antibody levels. Further studies of proinflammatory cytokines as modulators of latent CMV infection in older people in relation to cognitive decline are warranted.

There are some data indicating that Latino individuals experience a higher burden of Alzheimer's disease and an earlier onset of symptoms,[56–58] although it has been difficult to make conclusions regarding these findings because of differences in ethnic composition of Latino groups and because of the potential for ascertainment and selection biases in racial/ethnic comparative studies.[59,60] Further research is needed to assess whether latent infection and increased burden of inflammatory cytokines may explain the observed higher rates of cognitive decline in Latinos than in non-Latino whites, while taking into consideration variability in ethnic composition within study groups.

Prior HSV-1 and CMV infection are patterned by education and income.[19,20] There was a significant association between education and income and high CMV and HSV-1 antibody levels in the SALSA participants. Age and education were also significant predictors of cognitive decline. Therefore, age and education and their interaction with time were controlled for in all final models of cognitive decline. The education and income distribution of the SALSA participants was relatively homogenous, with most individuals reporting 4 to 11 years of education and an income of less than $1,000 per month.

Several factors have been identified as initiators of reactivation of latent herpesviruses, including age, chronic health conditions, depression/psychosocial stress, and exposure to ultraviolet light.[61–66] Of these factors, age, chronic conditions, and depression/psychosocial stress have been linked to cognitive decline in older people.[67,68] Correspondingly, the statistical analyses identified age and chronic health conditions as factors that were associated with higher CMV antibody levels and were also predictive of cognitive decline. Therefore, these variables were controlled for in all of the final models. There is also evidence that depression/psychosocial stress may influence risk of dementia through complex sociobiological pathways.[68–70] Various markers of psychosocial stress have been correlated with downregulation of specific markers of cellular immune functioning, including reactivation of latent CMV infection.[71–73] The assessment of depressive symptoms as a measure of psychosocial stress in the SALSA participants was not significantly associated with high CMV IgG antibody levels. In addition, depressive symptoms were not associated with a statistically significant rate of cognitive decline. These findings are consistent with other studies.[74,75] It has been suggested that depression may be cross-sectionally associated with cognitive impairment but does not significantly predict rate of cognitive decline.[74,75] It is possible that psychosocial stress measures other than depressive symptoms may be related to CMV reactivation and cognitive decline. Further studies are required to examine whether other measure of psychosocial stress, such as discrimination and absence of social support, influence the relationship between CMV antibody levels and cognitive decline.

A few factors may have reduced the ability to detect a significant association between HSV-1 antibody levels and cognitive decline and may have also resulted in an underestimation of the relationship between CMV and rate of cognitive decline. For example, the study population showed a moderate practice effect over the 4-year period, which led to an annual increase in 3MSE scores. Regardless of the presence of this practice effect, participants with high CMV antibody levels showed a significantly faster rate of cognitive decline. In addition, the analyses were limited to subjects who had participated in the study for at least 2 years starting from baseline. This resulted in the exclusion of study participants with higher risk profiles for cognitive decline, including older age and lower levels of education and income. Therefore, the analysis subsample most likely represents participants with generally healthier risk profiles and may have resulted in underestimation of the relationship between the viral antibody measures and cognitive decline.

It was not possible to examine the relationship between infection and specific forms of cognitive decline. For example, earlier research has shown that CMV DNA is found in a higher proportion of the brains of people with vascular dementia than in age-matched controls,[35] and several studies have implicated CMV infection as a risk factor for cardiovascular disease outcomes.[76] Research aimed at assessing whether CMV antibody titers are specifically related to risk of cognitive decline associated with vascular dementia is needed.

CONCLUSION

In summary, this is the first study to suggest that high levels of antibody to CMV are associated with a more-rapid decline in global cognition in a population-based sample of elderly individuals in the United States. Because multiple social and pathophysiological changes most likely influence cognitive decline, an assessment of several markers and

varying patterns of these markers, including infection, cellular immune response, inflammatory processes, endocrine levels, and genetic and social factors will provide the most insight into mechanisms of cognitive decline in elderly populations. The findings reinforce the need for additional large-scale prospective investigations to determine the influence of CMV antibody levels on rate of development of cognitive impairment or dementia, as well as a broader understanding of the potential mechanisms by which these viral immune measures may affect cognition. Confirmation of these findings will enhance understanding of whether CMV infection is a causative factor and may affect interventions aimed at targeting infection or viral reactivation over the life-course.

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Author Contributions: A. E. Aiello conceived the aims of the study, conducted the statistical analyses, and wrote the manuscript. M. N. Haan created the SALSA study and contributed to the design, concepts, and writing of the manuscript. W. Jagust contributed to the concepts and writing of the manuscript. J. Gonzalez assisted with model building and statistical analyses of the data. K. Moore aided in organizing the data and biostatistical analyses. L. Blythe coordinated the laboratory testing and organization of the data.

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